

DIAPAUSE AND PARTHENOGENESIS IN THE EGGS OF THREE
SPECIES OF PHASMATODEA.

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(Plate x, B; one Text-figure.)

[Read 29th November, 1961.]

Synopsis.

Podacanthus wilkinsoni Macl., *Didymuria violescens* (Leach) and *Ctenomorphodes tessulatus* (Gray) are stick insects (Phasmatodea) which have occurred recently in plague numbers in some eucalypt forests in south-eastern Australia.

These three species have been recorded as having both a one-year and two-year life cycle in the field, and experiments were designed to study their embryogenesis, and the occurrence of embryonic diapauses indicated by field observations. The eggs of each species may have one or two diapauses. Where there is one diapause in eggs of *P. wilkinsoni* and *D. violescens* it is post-embryonic and either pre-embryonic or post-embryonic in eggs of *C. tessulatus*. In those eggs which have two diapauses, the first diapause is pre-embryonic, variable in duration, and determines the length of the life cycle. The second diapause is post-embryonic.

It appears that eggs of the two highland species, *P. wilkinsoni* and *D. violescens*, require preliminary exposure to periods of cold for morphogenesis and hatching to occur. *C. tessulatus* occupies a warmer climatic region than the other two species and its eggs do not require the same intensity of cold during diapause for morphogenesis and emergences to occur.

P. wilkinsoni and *D. violescens* in the Jenolan area have predominantly a two-year life cycle when the eggs are kept in an environment normally occupied by the species and also when kept in a coastal environment. *C. tessulatus* in the Kempsey area has predominantly a one-year life cycle. Thelytokous parthenogenesis was observed in *P. wilkinsoni* and *D. violescens*, whereas deuterotokous parthenogenesis occurred in *C. tessulatus*. Females of *C. tessulatus* may retain viable sperms for at least ten weeks and those of *P. wilkinsoni* and *D. violescens* for at least one week.

INTRODUCTION.

Podacanthus wilkinsoni Macl., *Didymuria violescens* (Leach) and *Ctenomorphodes tessulatus** (Gray) are stick insects which have occurred recently in plague numbers in some eucalypt forests in south-eastern Australia. The general biology of *P. wilkinsoni* and *D. violescens*, the two species which occur mainly in the highland areas, has been described by Richards (1952) and Campbell (1960). The ecology of *C. tessulatus*, a pest in north-eastern New South Wales, was studied by Hadlington and Hoschke (1959).

The life histories of the three species are similar, although the duration of the life cycles is variable. Oviposition occurs during the late summer and autumn and the eggs hatch either during the spring of the same year or that of the year following oviposition. Individuals reach the adult stage and begin reproduction in mid-summer. A predominantly one-year life cycle for *C. tessulatus* was established by Hadlington and Hoschke (1959), while Richards (1952) and Campbell (1960) recorded a predominantly two-year life cycle for *P. wilkinsoni* and *D. violescens*. Campbell (1960) found that, by sampling egg populations in the field, a reliable assessment could be obtained of the numbers of nymphs which hatched from these in the following seasons. From his work, it is apparent that the results would be more accurate after embryonic development in the eggs was complete. Observations made during his work suggested that an embryonic diapause was occurring in the eggs of these phasmatids and that a knowledge of the occurrence, intensity and duration of the diapause would be an essential preliminary to any studies of egg parasitism and other aspects of the ecology which involved the egg stage. The investigations recorded in this paper are intended to form a basis for a more detailed study of diapause and the factors which influence it.

* As noted by Key (1960), the correct form of the name is *tessulatus*, not *tessulata*, which was used by Key (1957), the generic name having to be treated as masculine.

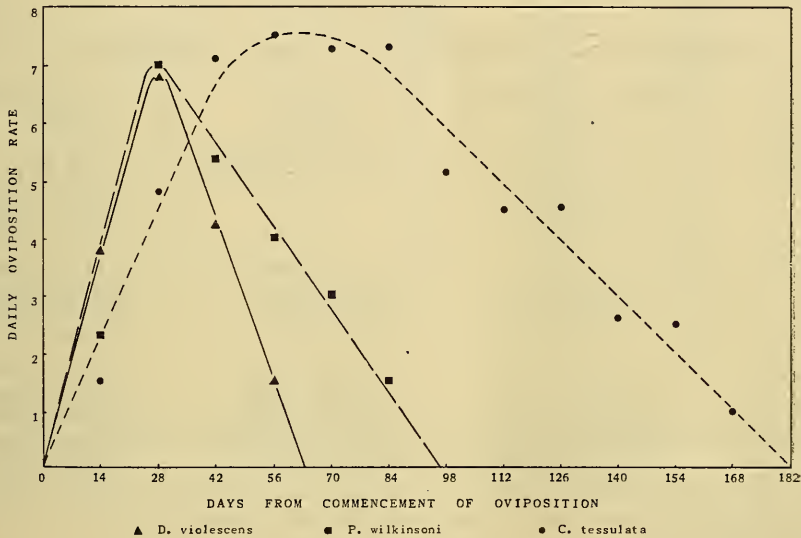
Parthenogenesis, which occurs frequently in the Phasmatodea, has been recorded for *C. tessulatus* and the investigation into diapause was extended to include the development of unfertilized eggs and to determine the extent to which parthenogenetic reproduction occurs in these three species.

MATERIALS AND METHODS.

(a) Procedure for Obtaining Eggs.

Final instar nymphs of *P. wilkinsoni* and *D. violescens* were collected in the field from the Jenolan area in the Central Highlands during December, 1957, and kept in cages at Hurstville during oviposition. Final instar nymphs of *C. tessulatus*, the progeny of insects collected in 1956 from Tanban State Forest near Kempsey, were also kept at Hurstville. Throughout this period the insects were fed on *Eucalyptus andreana* Naud.

Fertilized eggs of each species were obtained from females which were kept in cages with males. These females have been described hereafter as mated females,



although it is possible, but very unlikely, that some had not mated. Eggs of each species were also obtained from females which had no contact with males and these eggs were used to study parthenogenesis. Such individuals have been referred to as unmated females. The total number of females used is shown in Table 6.

Mated females of the three species commenced to oviposit 12 days after the final moult and unmated females 15 days after. The average number of eggs produced by each female, both mated and unmated, was 257 for *P. wilkinsoni*, 206 for *D. violescens* and 691 for *C. tessulatus*. The daily oviposition rates when plotted against the average age of the respective females has been expressed in Text-figure 1.

(b) Procedure for the Treatment of Eggs.

Eggs were collected each week and separated into four groups, each group being eggs laid during a particular period of oviposition, as shown in Table 1.

These eggs were placed in moistened sterilized sand in round plastic containers. The depth of sand did not exceed one inch and moisture was added each month. These eggs were kept at Hurstville where they were exposed to environmental temperatures. Some eggs from mated females of each species were kept at Hurstville in plastic jars in the absence of sand to determine whether hatching or embryonic development was

affected by soil moisture. Maximum and minimum temperatures were recorded daily and these have been expressed in Table 2 as mean monthly maxima and minima for the holding period.

Eggs of the three species of the same parental origin as previously mentioned, but not divided into groups representing the various oviposition periods, were also similarly prepared and kept at a constant temperature of 76° F. Light was excluded from all eggs during the holding period.

Eggs of the three species, kept in the field in environments from which the parents were collected, were destroyed shortly after the initiation of the investigation and provided no worthwhile data.

(c) *Examination of the Embryos.*

Egg samples were taken each month from the containers, placed in fixative and later dissected. Some eggs were stained to distinguish early embryonic development. From such examinations it was possible to study the occurrence and duration of the diapauses and the progress of morphogenesis in the egg.

TABLE 1.
Emergence Periods in Relation to Oviposition—Mated Females—(Coastal Environment).

Species.	Period of Oviposition (1958).	First Hatching (1958-59).	Second Hatching (1959-60).
<i>P. wilkinsoni</i> ..	1 Jan.-15 Jan.	6 Sept. (1 emergence)	9 Aug.-25 Oct.
	15 Jan.- 7 Feb.	27 Sept. (1 emergence)	20 Aug.- 8 Oct.
	7 Feb.-28 Feb.	—	17 Aug.- 8 Oct.
	28 Feb.-28 Mar.	—	15 Aug.-29 Sept.
<i>D. violescens</i> ..	1 Jan.- 8 Jan.	1 Nov. (1 emergence)	9 Sept.-30 Dec.
	8 Jan.-22 Jan.	15 Sept.- 5 Jan.	30 Aug.-18 Jan.
	22 Jan.- 7 Feb.	19 Sept.- 7 Nov.	22 Aug.- 1 Feb.
	7 Feb.-28 Feb.	25 Sept.- 8 Dec.	24 Aug.- 4 Jan.
<i>C. tessulatus</i> ..	1 Jan.-22 Jan.	4 Aug.- 4 Nov.	28 Aug.-19 Sept.
	22 Jan.-21 Feb.	13 Aug.-18 Dec.	26 Aug.-29 Sept.
	21 Feb.-21 Mar.	24 Aug.-22 Nov.	6 June- 5 Oct.
	21 Mar.-18 April	27 Aug.-31 Dec.	9 Aug.-11 Oct.
	18 Apr.-16 May	22 Nov.-18 Jan.	11 Aug.- 3 Nov.
	16 May-13 June		2 May-16 Sept.

(d) *Hatching.*

First instar nymphs were removed from the containers, and emergences from those eggs kept at the coastal environment were counted and sexed. Hatching occurred mainly during the early mornings (1 a.m. to 6 a.m.) for all eggs kept at the coastal environment temperatures, while nymphs of *C. tessulatus*, which emerged from eggs at 76° F., appeared at all times and hatching was not restricted to any particular period of the day. The months during which hatching occurred are given in Tables 1 and 2.

In the absence of added moisture, emergences of *C. tessulatus* did not occur, while in *P. wilkinsoni* and *D. violescens* the percentage hatch was lower than that for eggs held in moist sand. A large number of nymphs of *P. wilkinsoni* and *D. violescens* were unable to detach their metathoracic legs from the chorion.

RESULTS.

Parthenogenesis and Sperm Storage.

In the Phasmatodea parthenogenesis occurs frequently and in some species whose reproduction is entirely parthenogenetic the male is unknown. It was anticipated that parthenogenesis would occur in the three species studied since parthenogenetic, as well as sexual reproduction, had been recorded for *C. tessulatus* by Hadlington and Horschke (1959).

TABLE 2.
Emergences in Relation to Temperature.

Month and Year.	Hurstville Temperatures.		Emergences (% of Total Eggs).			Emergences (% of Total Eggs). <i>C. tessulatus</i> at 76° F. from 1/5/58 (1,110 Eggs).
	Mean Monthly Maximum.	Mean Monthly Minimum.	<i>P. wilkinsoni</i> (1,400 Eggs).	<i>D. violescens</i> (1,300 Eggs).	<i>C. tessulatus</i> (2,350 Eggs).	
February, 1958 ..	80	64	Nil	Nil	Nil	—
March	80	63	Nil	Nil	Nil	—
April	74	60	Nil	Nil	Nil	—
May	72	56	Nil	Nil	Nil	Nil
June	64	49	Nil	Nil	Nil	1.1
July	62	46	Nil	Nil	Nil	0.8
August	65	50	Nil	Nil	16.3	0.6
September	65	54	0.1	2.5	13.1	2.6
October	74	57	Nil	2.8	16.3	27.6
November	78	63	Nil	0.5	10.5	17.2
December	76	65	Nil	0.1	1.4	17.8
January, 1959 ..	79	67	Nil	0.1	Nil	2.2
February	80	68	Nil	Nil	Nil	0.3
March	78	64	Nil	Nil	Nil	1.3
April	74	61	Nil	Nil	0.1	1.3
May	68	52	Nil	Nil	Nil	0.2
June	64	48	Nil	Nil	Nil	0.5
July	64	46	Nil	Nil	0.1	0.1
August	66	48	10.1	0.2	6.2	0.3
September	69	55	33.2	13.0	12.8	—
October	72	56	0.7	6.2	0.6	—
November	75	64	Nil	4.5	0.1	—
December	78	64	Nil	2.5	Nil	—
January, 1960 ..	83	68	Nil	0.4	Nil	—
Total emergence	44.0%	32.8%	77.5%	72.6%

Eggs from unmated females of *P. wilkinsoni* and *D. violescens* yielded female individuals only, thus establishing thelytokous parthenogenesis for these species. In *C. tessulatus* deuterotokous parthenogenesis was recorded. Eggs from unmated females of *C. tessulatus* were collected at approximately monthly intervals over a period of six months and each group of eggs was held separately. There was a progressive increase in emergence numbers from eggs laid from the initial to the final oviposition period. Males emerged from eggs of the last oviposition period (Tables 3 and 4).

Field observations indicated that males and females were present in approximately equal numbers. The proportion of male and female progeny from mated females did not deviate significantly from the 50:50 sex ratio of males to females as shown in

TABLE 3.
Viability of Sperms—C. tessulatus.

Indicated by Proportion of Males in the Progeny from Eggs of Various Oviposition Periods. Expressed as percentage of total number of eggs.

Oviposition Period (1958).	Emergences—1958.			Emergences—1959.			Total 1958 and 1959.
	Males.	Females.	1958 Total.	Males.	Females.	1959 Total.	
1 Jan.—22 Jan. ..	44.5	39.0	83.5	2.0	1.5	3.5	87.0
22 Jan.—21 Feb. ..	43.0	39.0	82.0	2.2	3.2	5.4	87.4
21 Feb.—21 Mar. ..	41.4	37.6	79.0	4.6	4.6	9.2	88.2
21 Mar.—18 April ..	31.4	31.0	62.4	7.6	9.6	17.2	79.6
18 Apr.—16 May ..	9.0	6.6	15.6	29.2	24.0	53.2	68.8
16 May—13 June. ..	0.0	0.0	—	10.0	18.0	28.0	28.0

* No males survived after this date.

Table 5 and it appeared that the usual method of reproduction was sexual rather than parthenogenetic for these three species.

Females of *P. wilkinsoni* and *D. violescens*, which had been kept with males and then isolated for a period of one week, produced eggs at the end of this period of isolation, both male and female nymphs hatching from these eggs. When the same females were dissected after nineteen days' isolation, no viable sperms were found in

TABLE 4.
Parthenogenesis (C. tessulatus) and the Incidence of Male Progeny in Relation to Oviposition.
(Percentage of total eggs.)

Oviposition Period (1958).	First Emergence (1958).		Emergence (Males and Females).	Second Emergence (1959).		Emergence (Males and Females).	Total Emergence
	Males.	Females.		Males.	Females.		
1 Jan.-22 Jan. ..	0.0	1.0	1.0	0.0	0.5	0.5	1.5
22 Jan.-21 Feb. ..	0.0	2.0	2.0	0.0	1.4	1.4	3.4
21 Feb.-21 Mar. ..	0.0	0.8	0.8	0.0	4.2	4.2	5.0
21 Mar.-18 Apr. ..	0.0	0.4	0.4	0.0	8.0	8.0	8.4
18 Apr.-16 May ..	0.0	0.4	0.4	0.0	13.2	13.2	13.6
16 May-11 July ..	0.0	0.0	0.0	18.5	25.0	43.5	43.5

the spermathecae. Eggs laid by *C. tessulatus* females which had been in contact with males and then isolated for a period of ten weeks yielded male and female nymphs (Table 3).

The ability to store sperms thus occurred in the three phasmatid species, but was more pronounced in *C. tessulatus* than in the other two species. In high density populations the storage of sperms would not appear to be advantageous, but if the population was low and the chances of a female contacting a male less, then fertilization

TABLE 5.
Sex of Progeny from Mated and Unmated Females.
(Percentage of total emergences.)

Species.	Eggs from Mated Females.						Eggs from Unmated Females.					
	First Emergences.		Second Emergences.		Total.		First Emergences.		Second Emergences.		Total.	
	Fe- Males.	males.	Males.	fe- males.	Males.	fe- males.	Males.	fe- males.	Males.	fe- males.	Males.	fe- males.
<i>P. wilkinsoni</i> ..	50	50	55.4	44.6	55.4	44.6	—	—	—	100	—	100
<i>D. violescens</i> ..	44.8	55.2	49.3	50.7	48.4	51.6	—	100	—	100	—	100
<i>C. tessulatus</i> ..	52.4	47.6	50.0	50.0	51.7	48.3	—	100	17.4	82.6	15.9	84.1

of eggs by stored sperms may be important. The storage of sperms would ensure that eggs, produced later in the life of the female, were fertilized, particularly since the female appears to live much longer than the male (Table 6).

Degeneration was greater in eggs from unmated females than in those from mated females (Table 7).

Embryogenesis.

The morphological development of embryos and the differentiation of the various structures have been described by Steele (1941) in her study of the embryonic behaviour of *Austroicetes cruciata* (Sauss.) and these aspects have not been considered in detail in this paper. Development and the movements of the embryos during embryogenesis appeared to be similar in all three species so that in describing the relevant aspects of embryogenesis the species are considered as one.

The embryo was first visible as a small plate-like structure in the region of the micropyle. The developing embryo, with its dorsal surface directed inwards towards the yolk, then moved posteriorly along the micropylar area towards the operculum during anatrepsis and in the opposite direction around the posterior pole during the early stages of catatrepsis. Throughout this development, the embryo was slightly folded. Towards the completion of catatrepsis it almost entirely occupied the chorion, the head being located at and facing the operculum, while the dorsal surface of the embryo lay along the micropylar area (Plate x, B). When in an advanced stage of

TABLE 6.
Adult Longevity—Coastal Environment.

Species.	No. of Insects.	Maximum (Days).	Average (Days).
<i>P. wilkinsoni</i>			
Female	27	130	80
Male	11	85	61
<i>D. violescens</i>			
Female	17	95	57
Male	11	61	42
<i>C. tessulatus</i>			
Female	15	210	147
Male	14	74	60

catatrepsis the embryo was pigmented, and the mandibles were strongly chitinized. Once embryogenesis was initiated, it was continuous for 12–14 weeks until late catatrepsis.

The stage prior to the formation of the platelet was not observed. Embedding and sectioning the eggs to study the nuclei were not successful and it was not determined at what point the diapause occurred prior to the appearance of the platelet.

The stage prior to the formation of the platelet is here referred to as “pre-embryonic” and is the stage at which the first diapause supervened in those eggs which had two diapauses. In this regard Lees (1955) recorded that no species was known in

TABLE 7.
Emergences and Embryonic Conditions of Eggs—Coastal Environment.
(Expressed as percentages of total eggs.)

Species.	From Mated Females.				From Unmated Females.			
	Emergences.	Dead Embryos.	Un-developed Eggs.	Diseased.	Emergences.	Dead Embryos.	Un-developed Eggs.	Diseased.
<i>P. wilkinsoni</i>	44.0	2.8	44.7	8.5	1.4	1.7	77.7	19.2
<i>D. violescens</i>	32.8	0.6	54.0	12.6	1.2	0.2	50.4	48.2
<i>C. tessulatus</i>	77.5	2.4	12.7	7.4	10.4	3.0	74.1	12.5

which an embryonic diapause supervened before the formation of the blastoderm, but Voy (1954), while studying the embryonic behaviour of *Clonopsis gallica* Charp. (Phasmatodea), recorded a non-developmental stage prior to visible embryogenesis and regarded this as preblastodermic. Apparently he could not be more precise about the occurrence of the diapause with respect to the nuclei.

In those eggs which hatched during the first year, the first diapause occurred when the embryo was fully formed so that this is here referred to as “post-embryonic”. In eggs which had two diapauses, the second was a post-embryonic diapause.

These periods of suspended development coincide with the winter months when lower temperatures prevailed, while morphogenesis occurred during the summer and autumn months, usually during January–April when higher temperatures occurred.

The Occurrence and Duration of the Diapauses.

One or two diapauses, namely, pre-embryonic and post-embryonic, occurred in the eggs of all three species of phasmatids, but varied in their duration. The variability in the duration of the pre-embryonic diapause determined the length of life cycle.

The eggs of the three species hatched during the first, second and third years. However, only one-year and two-year life cycle eggs have been expressed quantitatively. Individuals having a three-year life cycle, while hatching, were not recorded and are included in the totals of undeveloped eggs in the respective tables.

TABLE 8.
Diapauses in Relation to One-, Two- and Three-Year Life Cycles.

Species.	One-year.	Two-year.	Three-year.
<i>P. wilkinsoni</i>			
First diapause	Post-embryonic.	Pre-embryonic.	Pre-embryonic.
Second diapause		Post-embryonic.	Post-embryonic.
<i>D. violescens</i>			
First diapause	Post-embryonic.	Pre-embryonic.	Pre-embryonic.
Second diapause		Post-embryonic.	Post-embryonic.
<i>C. tessulatus</i>			
First diapause	Pre-embryonic or Post-embryonic	Pre-embryonic	Pre-embryonic
Second diapause		Post-embryonic	Post-embryonic

The eggs of *P. wilkinsoni* having a one-year life cycle showed no indication of a pre-embryonic diapause, as the embryo commenced to develop soon after oviposition and progressed to a stage of advanced development when the post-embryonic diapause supervened. Hatching occurred from these eggs during the following spring. Two diapauses, namely, pre-embryonic and post-embryonic, occurred in eggs which produced two-year and three-year individuals.

The eggs of *D. violescens* appeared to behave similarly to those of *P. wilkinsoni*, although the proportion of one-year individuals was greater, as shown in Table 9.

TABLE 9.
Progeny from Mated and Unmated Females.

Species.	From Mated Females.						From Unmated Females.					
	First Emergence.		Second Emergence.		Total Emergence (%)	First Emergence.	Second Emergence.		Total Emergence (%)			
	Males.	Fe-males.	Males.	Fe-males.			Males.	Fe-males.				
<i>P. wilkinsoni</i>	1	1	340	274	44.0	—	—	—	18	1.4		
<i>D. violescens</i>	34	42	173	178	32.8	—	2	—	6	0.6		
<i>C. tessulatus</i>	713	649	237	237	77.5	—	20	37	185	10.5		

The eggs of *C. tessulatus* produced one-year individuals of two types, namely, those which have no pre-embryonic diapause and over-winter in the post-embryonic diapause stage and hatch in spring and those which have a pre-embryonic diapause and hatch in the late spring or summer suggesting the avoidance of, or a shorter period for, the post-embryonic diapause. The eggs of *C. tessulatus* with two-year and three-year life cycles have either one pre-embryonic diapause or a pre-embryonic together with a post-embryonic diapause. When only one diapause occurred hatching took place during the late spring or summer suggesting the avoidance of the post-embryonic diapause. The

possible avoidance or reduced period of the post-embryonic diapause was indicated when embryogenesis occurred in the egg samples examined during the spring months.

The period of oviposition influenced, to some extent, the production of one-, two- and three-year individuals as shown in Table 4. Eggs laid during the earlier oviposition periods yielded a large proportion of one-year individuals, while two-year individuals predominated from the later periods.

Diapause in eggs from unmated females of each species was similar to those from mated females, yielding one-, two- and three-year individuals, although the percentages were much lower (Tables 9 and 10).

Effects of Temperatures on the Embryonic Behaviour.

(a) Field Environment Temperatures.

Richards (1952) and Campbell (1960) during their investigations recorded the predominance of the two-year life cycle for *P. wilkinsoni*. To determine the existence and proportion of the one-year life cycle in the climatic region naturally occupied by this species, eggs of *P. wilkinsoni* were collected from cage-held females which had been collected at Jenolan and these eggs were kept in moist sand in plastic containers buried one inch below ground level at 4,200 feet in the Jenolan area. Only two-year individuals (76%) were recorded from these eggs. The percentage of eggs which developed and subsequently hatched was greater than that (44%) recorded from eggs kept in a coastal environment.

TABLE 10.
One-year and Two-year Life Cycles of the Three Phasmatid Species.
(Percentage of total eggs.)

Species.	From Mated Females.			From Unmated Females.		
	One-year.	Two-year.	Undeveloped Eggs.	One-year.	Two-year.	Undeveloped Eggs.
<i>P. wilkinsoni</i> ..	0.1	43.8	44.8	0.0	1.2	77.7
<i>D. violescens</i> ..	5.8	27.0	54.0	0.1	0.5	50.4
<i>C. tessulatus</i> ..	57.3	20.2	12.7	0.8	9.2	74.1

From dissection data for field-collected eggs from Nundle, Hanging Rock and Tuggolo State Forests in the Central Highlands, Campbell (1960) derived a figure of 20-30% embryonic development in eggs not parasitized. The low figure for embryonic development may be attributable to the large proportion of degenerated eggs which may have accumulated over several years, and thus represented more than one oviposition year.

Defoliation by *D. violescens* was recorded during alternate years from Bago State Forest in the Southern Highlands, thus suggesting predominance of the two-year life cycle. Campbell (1960), from his field data during his egg sampling work, was able to demonstrate that embryonic development had occurred in the December-February period and the eggs subsequently hatched during the following November and December, thus confirming the predominance of the two-year life cycle. Campbell recorded as much as 43% development in the eggs, but this figure may be greater as his material probably included degenerating eggs from previous oviposition years. As with *P. wilkinsoni* there was evidence which suggested that the development was higher for eggs in the field in a region naturally occupied by the species than when kept in a coastal environment.

A predominantly one-year life cycle was recorded for *C. tessulatus* (Hadlington and Hoschke, 1959), this being confirmed by laboratory results (Table 3). Individuals having a two-year life cycle appeared to occur in the field, but to only a minor degree. Both the natural environment of this species and the coastal environment at Hurstville are similar, and no marked deviation in the embryonic behaviour of this species was expected.

(b) Coastal Environment Temperatures.

The occurrence and relative proportions of the one-, two- and three-year life cycle individuals from eggs of the three phasmatid species kept at coastal environment temperatures has been given in Tables 9 and 10. A two-year life cycle predominated for *P. wilkinsoni* and *D. violescens* while a one-year life cycle predominated for *C. tessulatus*.

Embryogenesis, diapause and emergences of nymphs occurred at the coastal environment temperatures in all species, although the emergence of *P. wilkinsoni* and *D. violescens* nymphs was lower than that recorded from the field in the Central Highlands. Eggs of these two species do not appear to have received sufficient stimulus from the coastal environment; possibly the winter temperatures on the coast were not low enough and of sufficient duration during the first winter period to terminate the pre-embryonic diapause. However, many of these eggs remain viable until the following winter when the diapause may then be terminated, resulting in a three-year life cycle. Whether four-year individuals occur was not determined, but this possibility cannot be overlooked in any of the three species.

TABLE 11.
Monthly Average Daily Maximum and Minimum Temperatures for 30 Years.
(Weather Bureau data.)

Month.	Jenolan Caves.		Sydney.		West Kempsey.	
	Average Max.	Daily Min.	Average Max.	Daily Min.	Average Max.	Daily Min.
January	77.6	52.4	78.6	65.1	86.0	63.1
February	76.9	52.7	78.8	65.5	85.0	63.4
March	72.0	49.6	76.6	62.9	82.8	61.0
April	63.2	43.7	72.0	57.7	78.2	55.5
May	56.1	37.9	67.0	52.4	72.7	48.9
June	51.1	34.3	62.8	48.1	67.8	43.8
July	50.0	32.7	61.8	46.4	67.6	42.2
August	53.5	33.6	64.3	47.6	70.8	43.3
September	60.2	37.3	68.3	51.4	75.9	47.5
October	67.4	41.9	71.7	55.9	79.4	53.1
November	72.6	46.7	74.5	59.8	82.4	57.7
December	76.4	50.6	76.9	63.2	84.3	61.4

The emergence of *C. tessulatus* nymphs was greater than for the other two species, and this result would be consistent with the fact that the coastal environment temperatures at which these eggs were kept would more closely approach the temperatures of the climatic regions occupied by this species than the temperatures of the climatic regions occupied by *P. wilkinsoni* and *D. violescens* (Table 11).

(c) Constant Temperature.

P. wilkinsoni eggs kept at 76° F. did not hatch. Embryogenesis did not occur and the eggs deteriorated at this constant temperature. The temperature was not low enough to terminate the pre-embryonic diapause which would have permitted morphogenesis to be initiated.

D. violescens eggs kept at 76° F. also failed to hatch. However, in this species embryogenesis occurred and the embryo progressed to an advanced stage of catatrepsis and then degenerated. This temperature enabled the pre-embryonic diapause to be completed, allowing morphogenesis to occur, but it was not low enough to terminate the post-embryonic diapause and permit hatching.

C. tessulatus eggs kept at 76° F. hatched and emergences were not confined to any particular period of the day as with those eggs exposed to diurnal fluctuations. The eggs commenced to hatch one month after being exposed to 76° F., but the emergences were not related to the seasons. The total percentage emergence differed very little from that for eggs kept at coastal environment temperatures (Table 2). The constant

temperature of 76° F. enabled the pre-embryonic and post-embryonic diapauses to be completed, thus permitting morphogenesis and hatching to occur.

DISCUSSION.

From the experimental data it appears that temperature, as a component of the environment, influences the diapause and embryonic behaviour of eggs of the three phasmatid species. Exposure to low temperatures for a sufficient period appears to ensure the completion of diapause, while the next phase of the development is dependent on higher temperatures for the completion of embryogenesis.

The occurrence of diapause in the eggs of the three species, in the various climatic zones occupied by each, ensures that the eggs may over-winter for one, two or more years and regulates the occurrence of morphogenesis and hatching to the most favourable periods. The optimum exposure to low temperatures was not determined, but there is evidence which suggests that there is variability within the eggs to the duration of these exposure periods which are necessary to terminate the diapause.

In the three species studied the two highland species, *P. wilkinsoni* and *D. violescens*, may be expected to have a lower optimum temperature for the termination of diapause than that for the coastal phasmatid, *C. tessulatus*. Lees (1955) has previously considered this in suggesting that the warmer the climate of the region occupied by a species the higher will be the optimum temperature required for the termination of diapause. The diapause and embryonic behaviour of these phasmatids appear to support his remarks.

The terms "diapause stage" and "diapause development" (Andrewartha, 1952) are used in the consideration of the diapauses in these phasmatids. The first and second periods during which morphogenesis appears to have ceased are referred to as the pre-embryonic and post-embryonic diapause stages respectively, while diapause development is used for the periods of physiological development in progress during the diapause stages and which must be completed as a prerequisite for the initiation or resumption of morphogenesis.

After oviposition, eggs producing two- or three-year individuals enter the diapause stage and some physiological development occurs at the low temperatures. Unless the temperature range for diapause development is sufficiently low and sustained, the egg will degenerate or fail to be stimulated by the higher temperatures which occur later and are necessary for morphogenesis.

Although the occurrence of four-year or longer life cycles was not investigated, these may occur in the three species in particular environments.

P. wilkinsoni.

For termination of the pre-embryonic diapause, eggs of *P. wilkinsoni* apparently require to be exposed to periods of sufficient cold, in terms of duration and intensity, to allow diapause development to proceed. This permits morphogenesis to occur as soon as the temperature of the environment enters the temperature range for morphogenesis, which is not reached until December in the Jenolan area when the average daily maximum is 76° F. Once initiated, this development proceeds until the embryo is in late catatrepsis, at which time it enters the post-embryonic diapause stage. When post-embryonic diapause development has been completed the young phasmatids emerge. The lower limit of the temperature range for emergence appears to be lower than that for morphogenesis and would be somewhere between the average daily maximum of 60–65° F. which occurs in September in the Jenolan area, and during August on the coast, as shown in Table 11. This is confirmed by the emergences in the field and from eggs kept on the coast (Table 1).

When kept at a constant temperature of 76° F. no development occurred in the eggs, although this temperature was within the temperature range for morphogenesis. In eggs of this species, from the Jenolan area, a sufficient exposure to a relatively low temperature range is an essential preliminary to the initiation of morphogenesis.

Eggs in the post-embryonic diapause stage, which have been kept at 76° F. after embryogenesis is complete, do not hatch, but after being exposed to periods of cold they

hatch when placed at 76° F. The intensity and duration of cold during the pre-embryonic diapause stage appear to determine whether morphogenesis will be initiated. Eggs vary in their threshold of exposure to chilling, but it was evident that percentage of eggs in which development occurs was greater for eggs kept in the highlands than on the coast, and this appears to operate through temperature as shown in Tables 2 and 11.

The occurrence of a three-year life cycle has not been determined in the field in the Central Highlands, but it has occurred from eggs kept on the coast. This would seem to be due to the higher minimum temperatures and shorter periods of exposure to such temperatures on the coast, so that diapause development is not completed in any one season. If three-year individuals occur in environments where plagues have occurred they would be in small numbers.

D. violescens.

In this species a number of eggs developed without the pre-embryonic diapause or this diapause was of a very short duration. These eggs over-wintered in the post-embryonic diapause stage and yielded one-year individuals. The eggs which produce two-year and three-year individuals have the pre-embryonic and post-embryonic diapauses and react to the environment in a similar way to *P. wilkinsoni*.

When kept at 76° F. morphogenesis occurred without exposure to low temperatures, suggesting that this holding temperature was within the temperature range for pre-embryonic diapause development and also for morphogenesis. Emergences did not occur from these eggs, indicating that post-embryonic diapause was not terminated and the constant temperature of 76° F. was above the temperature range for post-embryonic diapause development.

While no results are available from field material of this species, the eggs may be expected to behave similarly to *P. wilkinsoni* in respect of the intensity and duration of cold when compared with eggs held in a coastal environment. Three-year individuals, if present in the field, would occur to a minor degree.

C. tessulatus.

In this species most eggs have only one diapause so that they over-winter in the post-embryonic diapause stage. In the eggs which produce two and three-year individuals, the pre-embryonic and post-embryonic diapauses occur during successive winters.

At 76° F. both pre-embryonic and post-embryonic diapause stages are completed, suggesting that this temperature was within the temperature range for pre-embryonic and post-embryonic diapause development and it was also within the temperature range for morphogenesis. All processes then operated at 76° F. so that emergences occurred at any time and were not confined to the periods after exposure to lower temperatures, although the seasonal production of individuals occurred from eggs kept at coastal environment temperatures. It may be noted from Table 11 that the average daily minimum temperature for Kempsey, where this species occurs in plagues, is lower than that for Sydney.

The most effective portion of the temperature range for the termination of diapause varies from one species to another (Hogan, 1960), and this appears to be related to the climatic zone occupied by each species. The warmer the climate of occurrence the higher are the optimum temperatures for the termination of diapause. The behaviour of *C. tessulatus* eggs, when compared with that of the other two species, appears to support this viewpoint, also expressed by other workers.

The period of oviposition appears to affect the production of one-year and two-year individuals in this species and this operates through the diapause stage. Eggs which were laid during the earlier oviposition period (January-March) were exposed to higher temperatures at which both pre-embryonic diapause development and morphogenesis would be completed, and such eggs over-wintered in the post-embryonic diapause stage. During the later oviposition periods (April-June) the temperature had fallen below that necessary for morphogenesis to be initiated, and these eggs over-wintered in the

pre-embryonic diapause stage. The effect of the oviposition period on the occurrence of the diapauses is masked by the incidence of parthenogenesis, but when the results in Tables 3 and 4 are compared it may be seen that the oviposition period is significantly related to the occurrence of the pre-embryonic diapause stage.

Acknowledgements.

The authors are indebted to Messrs. K. G. Campbell, K. M. Moore and L. H. Bryant of the Forestry Commission of New South Wales and to Mr. L. R. Clark, Division of Entomology, C.S.I.R.O., for their helpful criticism of the manuscript. The photograph is by Mr. R. Moulton, Forestry Commission of New South Wales.

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EXPLANATION OF PLATE X, B.

An embryo of *P. wilkinsoni* in a stage of late catatrepsis.